

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Task Force Paper On Cerebellar Transplantation: Are We Ready to Treat Cerebellar Disorders with Cell Therapy?

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1685699> since 2019-01-05T13:33:40Z

Published version:

DOI:10.1007/s12311-018-0999-1

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

Query Details

1. Please check if the affiliations are presented correctly.

ok

2. Presentation of section citations was modified in compliance with the journal style. Please check if all section citations are correctly captured.

ok

3. The abbreviation “L-Dopa” was changed to “l-DOPA” throughout the manuscript. Please check the provided expanded form “levodopa” if correct.

ok

4. Quotation marks not used to indicate uncommon or unusual usage at first mention or to indicate that a word or phrase is being referred to as a term rather than being used in its meaning were removed, and the terms were styled in italics. However, quotation marks used in quoted text(s) were retained. Please check the changes made if correct.

ok

5. Please check the “Funding Information” statement if correctly presented.

ok

6. Please provide complete bibliographic details for reference# [8 and 146].

Parmar M. Towards stem cell based therapies for Parkinson's disease. Development. 2018;145(1). pii: dev156117. doi: 10.1242/dev.156117.

Ref 146: article in press
doi: 10.2174/1570159X15666171114142422

Task Force Paper On Cerebellar Transplantation: Are We Ready to Treat Cerebellar Disorders with Cell Therapy?

Jan Cendelin, ^{1,2}

Annalisa Buffo, ^{3,4}

Hirokazu Hirai, ⁵

Lorenzo Magrassi, ^{6,7}


Hiroshi Mitoma, ⁸

Rachel Sherrard, ⁹

Frantisek Vozeh, ^{1,2}

Mario Manto, ^{10✉,11}

Email mmanto@ulb.ac.be

¹ Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University, alej Svobody 1655/76, 323 00 Plzen, Czech Republic 

² Laboratory of Neurodegenerative Disorders, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, alej Svobody 1655/76, 323 00 Plzen, Czech Republic

³ Department of Neuroscience Rita Levi-Montalcini, University of Turin, 10126 Turin, Italy

⁴ Neuroscience Institute Cavalieri Ottolenghi, Orbassano, 10043 Turin, Italy

⁵ Department of Neurophysiology and Neural Repair, Gunma University Graduate School of Medicine, 3-39-22, Maebashi, Gunma, 371-8511 Japan

⁶ Neurosurgery, Dipartimento di Scienze Clinico-Chirurgiche Diagnostiche e Pediatriche, Fondazione IRCCS Policlinico S. Matteo, Università degli Studi di Pavia, 27100 Pavia, Italy

⁷ Istituto di Genetica Molecolare – CNR, 27100 Pavia, Italy

⁸ Medical Education Promotion Center, Tokyo Medical University, Tokyo, Japan

⁹ IBPS, UMR8256 Biological Adaptation and Ageing, Sorbonne Université and CNRS, Paris, France

¹⁰ Department of Neurology, CHU-Charleroi, 6000 Charleroi, Belgium

¹¹ Service des Neurosciences, Université de Mons, 7000 Mons, Belgium

Abstract

Restoration of damaged central nervous system structures, functional recovery, and prevention of neuronal loss during neurodegenerative diseases are major objectives in cerebellar research. The highly organized anatomical structure of the cerebellum with numerous inputs/outputs, the complexity of cerebellar functions, and the large spectrum of cerebellar ataxias render therapies of cerebellar disorders highly challenging. There are currently several therapeutic approaches including motor rehabilitation, neuroprotective drugs, non-invasive cerebellar stimulation, molecularly based therapy targeting pathogenesis of the disease, and neurotransplantation. We discuss the goals and possible beneficial mechanisms of transplantation therapy for cerebellar damage and its limitations and factors determining outcome.

Keywords

Ataxias
Cerebellum
Cerebellar reserve
Neurotransplantation
Stem cells

Introduction (J. Cendelin, A. Buffo, and L. Magrassi)

Diseases and damage of the central nervous system accompanied by a substantial reduction of neuron number are not easy to treat. In many diseases, e.g., neurodegeneration, it is also difficult to prevent neuronal death. Functional deficits caused by a loss of neurons are often only partially reversible or even irreversible due to the limited regenerative capacity of the central nervous system.

Neurotransplantation might be a hopeful solution. However, it has not yet become a routine therapy for a wide spectrum of neurological diseases. The problems of neurotransplantation and stem cell therapy for nervous system damage have already been summarized by Rossi and Cattaneo in 2002 [1] and later in other reviews [2, 3, 4, 5, 6]. Despite intensive research, many of the limitations for clinical use in humans still persist or have on recently been overcome.

Transplantation is the transfer of cells, tissues, or organs from one place to another. In the case of neurotransplantation, immature cells must be grafted because mature neurons are very sensitive to damage during manipulations and would not be integrated in the recipient's tissue. On the other hand, stem cells, neural progenitors, and embryonic or fetal neuronal precursors are more resistant to manipulation, with some retaining capacity for proliferation and migration and, potentially, axon and dendrite growth and synaptogenesis that are crucial for functional integration into the host's neuronal circuits. Therefore, the development of neurotransplantation as a therapy profits from the huge progress in stem cell research and biotechnology progress during recent years.

The possibility to employ human neural stem cells for cell replacement therapy in neurodegenerative diseases has raised hopes in the field. Recent advancements in technologies allowing derivation, in vitro expansion, and specification of human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs) have achieved remarkable experimental outcomes, demonstrating that neuronal replacement is feasible and can attain behavioral recovery in several paradigms ([7, 8] and references therein). Benefits are particularly evident in preclinical models reproducing focal pathologies with a predominant loss of a single neuron subtype, such as substantia nigra pars compacta dopaminergic neurons in Parkinson's disease (PD) or striatal medium spiny neurons in Huntington's disease. Moreover, the pre-transplant multiplication of human multipotent/pluripotent stem cells overcomes the limited availability of human fetal neurons, which previously was the donor population that provided the most promising results, for instance in both preclinical and clinical settings of PD, although results were partly contradictory [9]. The progress of this field is testified by the prospect of clinical trials in PD patients employing human neurons derived from hESCs [10].

However, among the neurodegenerative pathologies, PD may represent an example particularly amenable to transplantation approaches, because motor symptoms of PD can be suppressed simply by secretion of dopamine by grafted cells with no need of precise synaptic integration of the graft. Thus, it is not clear yet whether neurotransplantation therapy would be of the same efficiency for other brain structures. Due to specific features of the cerebellum, such as low neurogenic potential, intrinsic limitations related to cerebellar circuitry, or poor knowledge on how to replicate cerebellar development with human cells [1], neurotransplantation therapy for its diseases seems to be still a distant goal and remains a big challenge [3]. Cerebellar transplantation has been investigated for many years in mouse models of hereditary cerebellar degeneration. Pilot studies in cerebellar mutant mice were done in the late 1980s [11, 12, 13] and the research continued with high intensity into the 1990s [14, 15, 16]. The original idea of neurotransplantation therapy was to substitute lost neurons, mainly Purkinje cells, with grafted ones. However, this is not the only mechanism how the graft can help and various mechanisms can be of different importance in specific situations as will be discussed here.

Goals of Cerebellar Transplantation (J. Cendelin)

When considering neurotransplantation therapy, we have to clarify what we expect from this treatment, what kind and what stage of the disease we are going to treat, and what mechanisms of the graft effect would be employed to achieve the goal. The graft could provide (1) new cells that would substitute lost ones, (2) trophic and metabolic support, (3) reversion of pathogenic factors in the tissue, and (4) fusion of grafted cells and intrinsic cells threatened by the degeneration. Another mechanism of graft effect can be delivery of lacking neurotransmitter by grafted cells like in the PD, but this is probably not appropriate for cerebellar pathologies.

The goal of the transplantation and importance of individual mechanisms of graft action would be substantially different in the case of completely developed pathological state when the neurons have already degenerated (post-traumatic states, post-ischemic states, advanced stages of cerebellar degeneration, etc.) compared to developing cerebellar damage when most of the neurons are still alive but are threatened by degeneration (namely early stages of cerebellar degeneration). It is also important to take into account the distribution of the cell loss since focal destruction of all cell types requires a different approach compared to diffuse but selective degeneration of a certain cell type or few types within the persisting tissue structure. In general, the goals of the transplantation therapy for cerebellar damage can be cell substitution, rescue of degenerating cells, and support of residual cerebellar function. These goals might be achieved via various mechanisms. Various types of grafts might have different applicability, advantages and disadvantages, or limitations in relation to individual goals (Table 1).

Table 1

Summary of advantages and disadvantages (positive and negative factors) and potential (expected or desired) effects of various cell types for therapy of cerebellar diseases

| Cell type | Positive | Negative | Expected or potential effects |
|--|---|--|---|
| Embryonic stem cells | <ul style="list-style-type: none"> – Good in vitro propagation – Totipotent | <ul style="list-style-type: none"> – Ethically problematic source – Necessity to induce differentiation into cerebellar-specific neuronal phenotypes | <ul style="list-style-type: none"> – Cell substitution? – Plasticity stimulation? |
| Induced pluripotent stem cells | <ul style="list-style-type: none"> – No ethical problems – Propagation as in vitro culture – Pluripotent | <ul style="list-style-type: none"> – Necessity to induce differentiation into cerebellar-specific neuronal phenotypes – Potential tumorigenesis due to artificial manipulation of cell differentiation mechanisms? | <ul style="list-style-type: none"> – Cell substitution? – Plasticity stimulation? |
| Carcinoma stem cells | <ul style="list-style-type: none"> – No ethical problems – Easy maintenance and propagation as in vitro culture – Effective in therapy of several neurological diseases in animal models | <ul style="list-style-type: none"> – Risk of potential tumorigenesis – Necessity to induce differentiation into cerebellar-specific neuronal phenotypes | <ul style="list-style-type: none"> – Cell substitution? – Plasticity stimulation? |
| Mesenchymal stem cells | <ul style="list-style-type: none"> – No ethical problems – In vitro propagation – Rescue of degenerating neurons – Compared to other graft types, mechanisms of the effects are relatively well known | <ul style="list-style-type: none"> – Poor neuronal differentiation – Long persistence of effects is not guaranteed | <ul style="list-style-type: none"> – Cell rescue – Anti-inflammatory effect – Metabolic support – Cell fusion – Plasticity stimulation |
| Neural stem cells (fetal, adult) | <ul style="list-style-type: none"> – In vitro propagation (limited) – Good neural differentiation – Rescue of degenerating neurons | <ul style="list-style-type: none"> – Necessity to induce differentiation into cerebellar-specific neuronal phenotypes | <ul style="list-style-type: none"> – Cell rescue – Metabolic support – Cell substitution? – Plasticity stimulation? |
| Embryonic (fetal) cerebellar cells | <ul style="list-style-type: none"> – Determined differentiation – Good source of cerebellar neuronal phenotypes | <ul style="list-style-type: none"> – Ethically problematic source – Low availability – No in vitro propagation | <ul style="list-style-type: none"> – Cell substitution? – Plasticity stimulation? |
| <p>In some cases, the same factor can represent both advantage and disadvantage depending on the particular situation. Most of the effects are rather hypothetical and speculative since direct evidences are lacking (indicated by “?”). Cell substitution is the original idea (and theoretically optimum goal) of neurotransplantation tested in all types of grafts, but in most of the grafts, it seems to be the less applicable mechanism. Plasticity (and/or regenerative processes) stimulation might be, on the other hand, a universal mechanism of neurotransplantation therapy effect. For details and references, see the text</p> | | | |

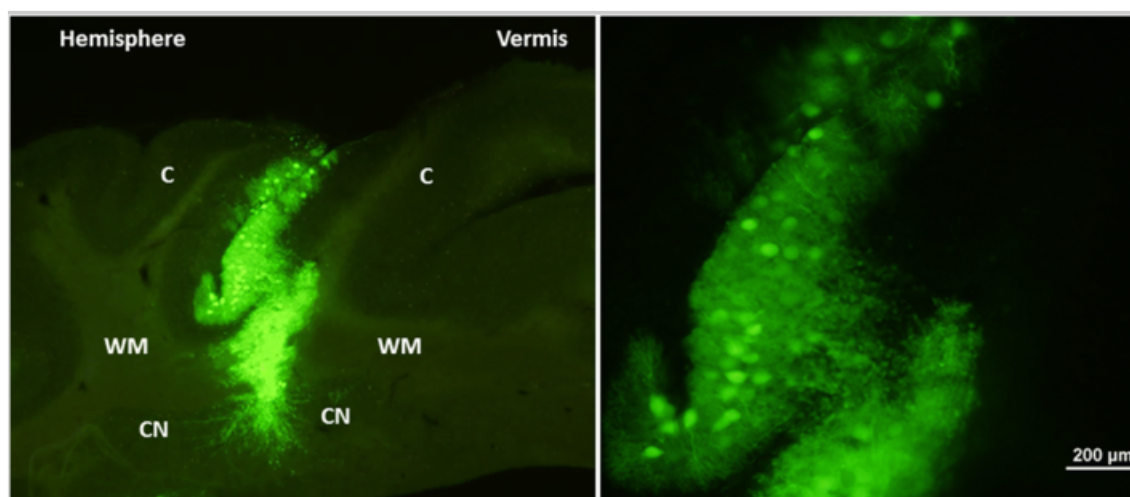
Cell Substitution

Cell substitution is the delivery of new cells by their grafting (or more precisely, grafting their precursors) aiming to replenish the number of cells of a given phenotype or several phenotypes that has been reduced due to the pathological process. If fully successful, this goal would be an optimal outcome of transplantation therapy for advanced cerebellar damage because it would, theoretically, restore the normal structure of the tissue and, thereby, cerebellar function.

This approach has been investigated many times in mouse models of cerebellar degeneration. Most of the studies focused on the analysis of graft survival, morphology, and presence of individual cerebellar cell phenotypes. These studies showed that embryonic or fetal cerebellar tissue is a good source of Purkinje cells [11, 12, 15, 17, 18, 19, 20] (Fig. 1), whereas various types of stem cells and neural progenitors mostly failed to differentiate into specific cerebellar neuronal phenotypes [21, 22, 23, 24, 25, 26]. Several studies also tested the impact of transplanting embryonic (fetal) cerebellar tissue, with functional integration being shown between climbing fibers and Purkinje cells or granule cells [13, 27, 28, 29, 30]. Among these studies, Triarhou et al. [31, 32] and Kaemmerer and Low [33] even reported improved motor performance in treated Purkinje cell degeneration (PCD) and spinocerebellar ataxia type 1 (SCA1) mice, respectively. Although one could speculate that in these cases reduced ataxia was potentially due to Purkinje cell substitution, direct evidence is lacking, and other mechanisms (such as trophic effects) might be involved. Furthermore, no (or only minor and inconclusive) improvements were seen in Lurcher [20, 34] and Tambaleante [35] mice after Purkinje cell precursor transplantation, suggesting that cell substitution is not always, if ever, efficient. Thus, substantial evidence of cell substitution within the cerebellum has not been proven even for grafts of embryonic or fetal cerebellar tissue, wherein cell differentiation is well reproduced.

Fig. 1

Mouse embryonic cerebellar tissue expressing enhanced green fluorescent protein (EGFP) that has been grafted into the normal mouse cerebellum (left). In this case, the grafted tissue (bright green) spreads through the cerebellar cortex (C) and white matter (WM) and reaches the cerebellar nuclei (CN). At higher magnification (right), graft-derived Purkinje cells (EGFP-positive) can be seen colonizing the host's tissue outside the graft mass



In summary, there are only few indices that this approach may be fully effective in mouse models of cerebellar degeneration and, currently, some data are rather pessimistic. Potential functional recovery through cell substitution would be essentially dependent on the delivery and survival of the sufficient number of cells, their differentiation into appropriate cerebellar cell types, as well as their migration, goal-directed axon and dendrite growth, and specific cell-to-cell synaptic connection. The achievement of all these goals does not appear to be an easy task.

Cell Rescue

Several studies have shown that grafted mesenchymal stem cells (MSCs) [36, 37, 38, 39], and in some cases neural stem cells [21, 26, 40], can prevent Purkinje cell death in mouse models of cerebellar degeneration (see paragraph 4). For this purpose, the therapy should be started in the early stages of disease before the cells are lost. Thus, this mechanism of graft effect could be employed in diseases for which precise diagnosis can be made in advance (mutation carriers) or in the early disease stages, such as for late-onset and slowly developing degenerative disorders. However, this mechanism of graft effect is not appropriate in cases of advanced, severe neuronal loss. This mechanism (cell rescue) of transplantation therapeutic effects seems to be the most efficient one providing functional improvement in several mouse models of cerebellar degeneration.

Support of Residual Cerebellar Function

After mild neuronal loss, cerebellar function could be maintained at an adequate level by activity of residual cerebellar tissue—cerebellar reserve [41]. More significant loss of tissue is manifested as decline of cerebellar function, indicating that the damage has exceeded cerebellar reserve potential [41]. Nevertheless, the activity of residual cerebellar tissue and, thereby, cerebellar reserve could be promoted by therapeutic intervention, of which neurotransplantation might be one possibility [6].

Grafted cells can improve neuronal function through their neurotrophic effects on degenerating neurons by secreting neurotrophic factors, supporting mitochondrial function and controlling neuroinflammation [21, 37, 40, 42]. By producing neurotrophic factors [37, 43, 44], grafted cells may also facilitate endogenous regenerative processes, enhance synaptic plasticity, and modulate synaptic function in the cerebellum [45, 46]. Indeed, replenishing cerebellar neurons would also increase cerebellar reserve and could turn the diseased cerebellum back to a state that provides adequate functional restoration—“the restorable stage” [6, 41]. This stage was defined by Mitoma and Manto [41] as a stage in which there is sufficient, effective residual cerebellar tissue that an appropriate therapy can restore or augment overall cerebellar function (see paragraph 7.2.). Thus, transplantation could, theoretically, augment function of the residual cerebellar tissue (by supporting cell function) as well as replenish residual cerebellar tissue (by cell substitution).

Transplantation of Stem or Embryonic Cells: Cell Migration, Differentiation, and Axon Growth (A. Buffo and L. Magrassi)

Adequate development and survival of stem or embryonic cells after their engraftment is important for therapeutic benefit. If the goal is to replace lost neurons and integrate grafted cells into the neural circuits, these factors are essential. To restore cerebellar function by grafting immature cells, cerebellar development should, in principle, be reproduced by the graft. This process can consist of an *in vitro* phase that would pre-determine stem cell differentiation but must be finalized *in vivo* in the graft recipient's cerebellum. Although studies using stem cells were rather disappointing (see paragraph 2), recent technological advancements and achievements in other fields hold new promises and point to novel issues to be solved.

Differentiation of Stem Cells

The potential of hESCs or hiPSCs to model the complex development of the cerebellum is just starting to be explored, with only a handful of studies to date that reported the generation of human Purkinje cells and other cerebellar neurons (mainly granule cells) [47, 48]. Early protocols attempted cerebellar differentiation from either mouse or hESCs by mimicking the *in vivo* signals inducing the cerebellar territory. However, the resulting cerebellar cultures only contained a small proportion of Purkinje cells and a relatively high number of cerebellar granule cells [49, 50, 51]. Muguruma et al. [52] adopted a different approach, based on reproducing cerebellar patterning, and exploited exposure of committed progenitors to the instructive action of granule cell precursors. This method resulted in the generation of

mouse Purkinje cells at a much higher efficiency. Higuera and collaborators [53] focused on obtaining an expandable population of cerebellar neuron progenitors from mouse ESCs that could then be differentiated in defined media to produce a considerable number of neurons with morphology, markers, and electrophysiological properties of mature Purkinje cells. As regards human cells, Wang et al. [54] applied a self-inductive approach following Muguruma et al. [52] on hiPSCs co-cultured with human fetal cerebellar slices. Although cells appeared to display Purkinje cell-specific markers and were electrically active, they did not possess the characteristic morphology of mature Purkinje cells. Most recently, the approach adopted in Muguruma et al. [52] was adapted to generate Purkinje cells from hiPSCs, achieving neurons with morphology and phenotype clearly resembling fully mature Purkinje neurons [55]. Moreover, the introduction of a selection step based on *Thy1* expression in combination with co-culturing with mouse granule neurons obtained 60–91% of quite mature Purkinje neurons [56]. Alternatively, hESC-derived organoids can be used to develop Purkinje cell differentiation [57], although the degree of Purkinje cell maturation achieved still requires full evaluation [57]. This cerebellar organoid protocol has recently been simplified to obtain human cerebellar progenitors from 3D hiPSC cultures, with efficiencies of up to 90%, an approach that leads to about 10% of maturing Purkinje neurons [58].

Careful design of efficient *in vitro* protocols producing correctly specified progenitors is especially crucial for the success of *in vivo* transplantation. Indeed, committed cells are far less sensitive than multipotent/pluripotent progenitors to the lack of proper neurogenic cues of the adult brain or to the presence of injury-derived anti-neurogenic signals [1, 8], which may result in failure of cell replacement attempts. Further, full control on differentiation steps is necessary to pinpoint the best stage for grafting, full commitment to the required cell type, and low sensitivity to dissociation and grafting procedures, which affect the survival of fully differentiated neurons. Thus, a significant research effort remains to be made to obtain consistent protocols capable of reproducibly generating large quantities of properly specified cerebellar neurons and further ensuring no contamination by tumorigenic or unwanted neural types, as well as no risk for genomic instability.

Survival of Grafted Cells

Additional challenges in any cell replacement approach reside in the capability of grafted cells to survive, differentiate, and integrate in appropriate territories and form correct connections to support lost functions in the context of the diseased tissue. The results of transplanting dissociated fetal mouse cerebellar neurons and mouse ESC-derived cells apparently indicated a rather low survival rate following implantation (collectively below 3% of total transplanted cells) [49, 50, 52, 53]. This was true even in the most favorable experimental conditions when the cells were grafted into the embryonic cerebellar anlage [59]. Moreover, there was no specific survival advantage of Purkinje cell precursors engrafted in mutants lacking endogenous Purkinje cells [49, 50, 52, 53]. However, the heterogeneous nature of the transplanted cells in terms of differentiation potential and survival ability makes these observations less relevant. Interestingly, transplantation of small fragments of the developing cerebellum in experimental models of Purkinje cell degeneration has shown that fetal cerebellar neuronal progenitors have a remarkable capacity to leave the transplant, colonize the deprived cerebellar cortex, mature as Purkinje neurons, and connect into host circuits [30]. In some cases, this was associated with mild behavioral improvement [60].

Problems in Differentiation, Survival, and Integration of Grafted Cells

Despite signs of behavioral improvement, in most studies, significant recovery of motor function remained hampered by the inability of most transplanted Purkinje cells to make connections with the host cerebellar nuclei (e.g., [30]). This limitation is not present if fetal cerebellar precursors [59], or Purkinje cell precursors obtained from mouse ESCs [52], are grafted during fetal cerebellar development. After transplantation into the developing cerebellum, dissociated Purkinje cell precursors of any origin matured into Purkinje cells, some of which were ectopic with a distorted dendritic arbor

[35, 52, 53, 59], but the majority of which established appropriate connections and correctly integrated in the host Purkinje cell layer where they could survive for the entire life span of the host [61]. However, while ectopic Purkinje cells transplanted during development may still receive and make appropriate connections with the cerebellar nuclei [59, 62], this capability clearly declines during developmental progression so that, after grafting into adult cerebella, while still expressing maturation markers, transplanted Purkinje cells display prominent aberrant orientations with dendrite abnormalities and no cortico-nuclear projections [35, 52, 53, 62]. Moreover, functional improvements achieved when Purkinje cell precursors are grafted into abnormal adult cerebella lacking Purkinje neurons remain controversial. Functional benefit has been reported in some studies [31, 32, 33], but others have not detected any, or only very mild, changes [20, 34, 35]. Also to date, no studies on ESC-derived cells have explored behavioral improvements after grafts in disease models. Taken together, with the currently used approaches of cerebellar neurotransplantation, correct integration of grafted cells appears problematic especially in the mature cerebellum. This somewhat diverges from studies focused on neurodegeneration models in other CNS areas where fetal neurons, or in vitro specified mouse and human ESC/iPSCs, not only survive, extend long-range projections to target areas in the injured adult brain, and connect into the correct circuits but also can improve behavior [63, 64, 65, 66, 67, 68, 69].

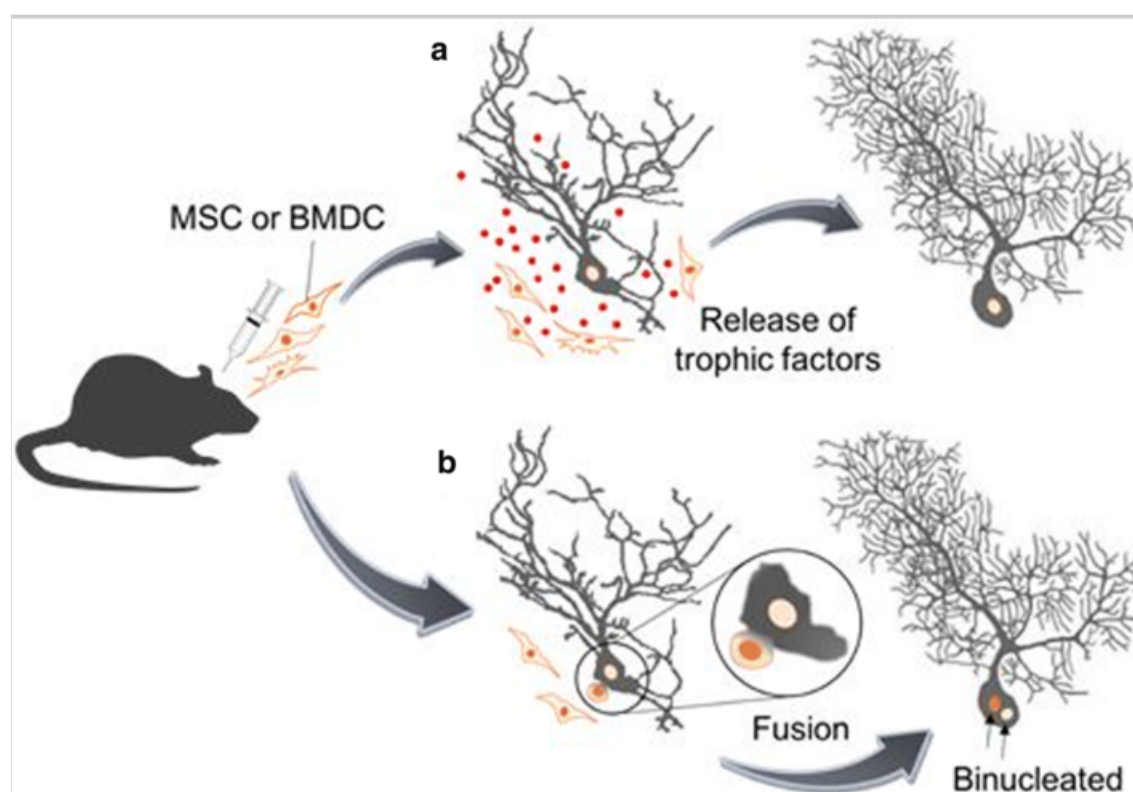
Difficulties with transplants of Purkinje cell precursors in the adult may be due to several factors. First, poor survival of transplanted cells may reflect the particular metabolic demands of cerebellar progenitors, specifically Purkinje neurons [53]. Second, the mature cerebellar environment appears particularly refractory to integration of new elements, despite some plastic changes being triggered in the adult parenchyma by exposure to immature elements [70]. Indeed, the mature granule cell layer is known to act as a barrier that prevents grafted Purkinje cells from migrating to the proper destination and their axons from growing toward the deep cerebellar nuclei [62]. Of the many cues hampering the correct distribution of cerebellar progenitors, reelin is a likely candidate [71, 72]. Of note, low cerebellar receptiveness is not always significantly altered or increased in pathology models [35, 52, 53], suggesting that disease does not necessarily worsen or facilitate integration of grafted cells. Thus, to promote a functionally relevant amount of graft integration, cell replacement strategies for cerebellar diseases could aim at preventive transplantation into the immature cerebellum before disease onset. This option may well apply to hereditary cerebellar ataxias, whose transmission permits prenatal diagnosis. However, a first study where mouse fetal progenitors were grafted in a genetic ataxia mouse model failed to show any preventative benefit of the graft [35]. Alternatively, strategies should be developed to neutralize adverse environmental factors, provided that abolition of cues stabilizing mature cerebellar architecture and/or connectivity does not impair the host's established circuits and functions. Third, low integration efficiency of grafted cells might also reflect poor or inappropriate differentiation protocols for human multipotent and pluripotent cells. Although more work is needed to develop more efficient in vitro strategies, this is unlikely to promote better integration per se, because integration defects also occur for primary cerebellar progenitor grafts [20, 34, 62, 73]. Fourth, the poor capability of grafted Purkinje cells to connect with cerebellar nuclei in the mature cerebellum may reflect the low propensity of neonatal rodent Purkinje neurons, compared to embryonic cells, to sustain axon extension in favor of axon arborization, a switch which occurs in association with exposure to granule cell-derived signals [74]. How the potential for axon extension can be enhanced in Purkinje progenitors before grafting remains to be understood. In summary, intensive efforts to create permissive in vivo conditions for the improved integration of grafted Purkinje neurons will be essential to achieve functional recovery by cell replacement in cerebellar degenerative disease. However, successful cerebellar grafts that will be able to establish connection with the host deep cerebellar nuclei could also worsen the subject's clinical condition due to a possible imbalance of activity between the grafted neurons and the surviving host cerebellar tissue. In humans, graft-related dyskinesias have been often described after fetal neural precursor transplantation for Parkinson's disease. Most of the subjects that developed graft-related dyskinesias displayed successful grafts surviving years after transplantation [75].

Rescue of Degenerating Neurons in Animal Models of Cerebellar Disorders by Transplantation of Stem Cells (H. Hirai)

In addition to providing new cells to replace those that have degenerated, cell transplantation can also provide factors to help maintain neurons in the process of degeneration. When tissues are injured by various types of insults such as infarction, trauma, and neurodegenerative diseases, affected cells release numerous cytokines and growth factors, which trigger local immune responses and chemotaxis of various cell types [76, 77]. Bone marrow-, adipose tissue-, and umbilical cord-derived MSCs are representative cell types that are attracted to the damaged tissues by the alerting signal molecules. The attracted MSCs help to mitigate local inflammation by releasing immunomodulatory and anti-inflammatory factors [78, 79, 80]. To date, many animal experiments have been performed to validate beneficial influence of MSCs on neurodegenerative diseases. For example, injection of MSCs intravenously to spinocerebellar ataxia type 2 (SCA2) transgenic mice [81] or directly into the cerebellar tissue of SCA1 transgenic [39] or new born Lurcher mutant [37] mice alleviated progressive degeneration of Purkinje cells, resulting in better behavioral performance compared with respective non-treated control mice. Likewise, injection of MSCs into the intrathecal space of SCA1 knock-in (KI) mice significantly reduced progressive degeneration of spinal motor neurons [82]. Similar to MSCs, transplantation of precursor neural stem cells (NSCs), which were derived from the subventricular zone of adult mice, into the cerebellar white matter of SCA1 transgenic mice also mitigated degeneration of Purkinje cells [25]. These results suggest that the MSCs and NSCs have potential to prevent affected neurons from degeneration and cell death. Although the cellular and molecular mechanisms behind the suppression of neurodegeneration have not been fully clarified, conditioned medium, in which MSCs were cultured, was shown to exert similar therapeutic actions as MSCs on degenerating spinal motor neurons of SCA1 KI mice [82], suggesting that treatment with beneficial factors secreted from the stem cells would be a feasible possibility (Fig. 2(A)).

Fig. 2

Schema depicting possible mechanisms that account for beneficial effects of cellular transplantation. Transplanted MSC and BMDC are attracted to degenerating neurons and release molecules trophic to the impaired neurons (A). Alternatively, the grafted cells fuse with degenerating neurons, resulting in binucleated neurons (B). MSC mesenchymal stem cell, BMDC bone marrow-derived cell



A different and intriguing possibility that accounts for beneficial effects of MSCs and bone marrow-derived cells (BMDCs) is the fusion of the injected cells with impaired neurons (Fig. 2(B)). Several studies demonstrated the fusion of grafted BMDCs [83] and MSCs [37, 84, 85] with degenerating Purkinje cells. The fused Purkinje cell is binucleated: the donor nucleus was proven to be reprogrammed to a Purkinje cell nucleus and begin to produce proteins selectively expressed in Purkinje cells such as calbindin D28K and Purkinje cell protein 2 (L7 protein) [86, 87]. With respect to the cellular fusion, whether the grafted cells fuse with healthy Purkinje cells or only with impaired Purkinje cells would be an intriguing question. This question was addressed in experimental models of incomplete Purkinje cell degeneration induced by specific neurotoxic treatments [88] or by genetic mutations [85]. The number of Purkinje cells fused to bone marrow-derived hematopoietic precursors was approximately 1 order of magnitude greater in mice and rats where Purkinje cells were damaged either by the intraventricular injection of propidium iodide or that of an immunotoxin targeting the p75NGF receptor, than in untreated animals [88]. The same question was also addressed by injecting MSCs into healthy wild-type mouse cerebellum and comparing the results with that obtained using symptomatic SCA1 transgenic mice [85]. Examination of the cerebellar tissues from healthy wild-type mice that received cerebellar injection of MSCs (50,000 cells/mouse) revealed only one MSC-fused Purkinje cell found in only one of 21 mice examined, which was in sharp contrast with high frequency of appearance of MSC-fused Purkinje cells in MSC-injected symptomatic SCA1 transgenic mice (158 Purkinje cells in nine mice out of 16 mice examined) [85]. These results show that MSCs have the potential to fuse primarily with impaired Purkinje cells. However, since the frequency of MSC-Purkinje cell fusion is very low, even in the degenerating cerebellum, it remains inconclusive whether the fusion event has substantial therapeutic significance.

Stability of grafted stem cells and persistence of the beneficial action on damaged/degenerative tissues have not been fully clarified, since many studies using model mice of cerebellar disorders examined the influence only within a couple of months after the engraftment [25, 37, 42]. However, two studies demonstrated morphological and behavioral improvements in SCA1 model mice 4 months or 6 months after the cerebellar or intrathecal injection of MSCs [39, 82]. Thus, the beneficial effects of grafted MSCs likely continue at least more than half a year, although the MSCs themselves may disappear earlier. The presence of grafted MSCs for only a limited period results in regression and eventual disappearance of the therapeutic benefits; however, even during a limited period, the local immunosuppression at acute-subacute stages should have marked therapeutic benefits for brain injuries such as ischemic insults [89]. Meanwhile, GFP expression in Purkinje cells that were fused with GFP-expressing MSCs was observed more than 6 months after the engraftment [85]. This suggests that donor nuclei of MSC origin are active, and therefore potentially therapeutic, in Purkinje cells for at least 6 months, much longer than the presence of the actual (MSCs) cells.

Role of Trophic Factors (R. Sherrard)

Trophic factors and other signaling molecules regulating survival, proliferation, differentiation, migration, fiber growth, and synaptogenesis are important for neurotransplantation for at least two reasons. First, they can be produced by grafted cells and rescue degenerating neurons [37], modulate pathological process in the residual cerebellar tissue (e.g., reduce inflammation), and hypothetically, also stimulate its function as discussed above. Secondly, trophic factors influence two major problems of cell transplantation for cerebellar disease and injury: survival of the grafted cells and their integration in the remaining cerebellar circuitry. Neuronal survival, neurite outgrowth, and synaptogenesis are processes in which trophic factors play major roles during development including that of the cerebellum [90] and thus could be a useful adjunct to cellular grafts.

However, production of neurotrophic factors by grafted embryonic or mesenchymal stem cells [44, 91] has a real possibility of facilitating integration of grafted neurons into established circuits, especially since they are extensively involved in the development of cerebellar neurons and the cortical circuit

[90]. The focus of cell transplant treatment is often the replacement of Purkinje cells [3], consistent with their central role in the cerebellar cortical circuit and regulation of deep cerebellar nuclear output. Unfortunately, as discussed in the section “Transplantation of Stem or Embryonic Cells: Cell Migration, Differentiation, and Axon Growth (A. Buffo and L. Magrassi),” there is very little success in integrating grafted Purkinje neurons into the existing cerebellar circuitry [62] and this is where neurotrophic factors may help because they are involved in important stages of Purkinje cell development that impact on its connectivity.

AQ2

Purkinje Cell Output to the Deep Cerebellar Nuclei

During maturation, Purkinje cells lose their capacity to regenerate the central part of their axon to restore connectivity to their deep nuclear targets [92]. However, even grafted immature Purkinje cells or progenitors cannot penetrate the environment of the mature internal granular layer to innervate their target, the deep cerebellar nuclear neurons [62], in contrast to grafted ESCs in other CNS regions, which do extend their axons to their targets [69]. This lack of grafted Purkinje cell axonal extension may be less due to a hostile cerebellar environment than the absence of an appropriate growth program. In the natural sequence of cerebellar development, rather than project an axon to the deep nuclei, immature Purkinje cells migrate through them leaving behind their axons in synaptic contact with their target deep nuclear neurons [93]. While grafting Purkinje cells adjacent to the deep nuclei may facilitate their reconnection, the Purkinje cell itself would then have to migrate to its normal location in the cortex in order to be able to receive its normal input, parallel and climbing fiber axons. While extracellular molecules such as reelin and tenascin are primary regulators of early Purkinje cell migration [94], the neurotrophic factor GDNF, and its receptor GFR α 1 have recently been implicated in Purkinje cell migration [95], which may offer future potential to overcome the current inability to establish effective graft Purkinje cell to host deep nuclear connectivity.

Purkinje Cell Dendritic Development and Afferent Synaptogenesis

Even if grafted Purkinje cells are placed in, or migrate to, the cerebellar cortex, their integration, and therefore therapeutic usefulness, requires that they develop a dendritic tree to establish and maintain afferent parallel and climbing fiber synapses. Neurotrophic factors are involved in both of these processes.

The development of the Purkinje cell dendritic tree is regulated by many factors but is principally an inherent growth program involving extensive calcium signaling pathways [96, 97]. However, trophic factors such as IGF-1 promote Purkinje cell dendritic growth [98, 99] and BDNF is particularly important for their spinogenesis, synaptogenesis, and synaptic function [45, 100].

Granule cells express both BDNF and its receptor TrkB throughout life [101, 102] and therefore retain the capacity for structural as well as functional synaptic plasticity. For example, in the presence of unoccupied Purkinje cell spines, such as those that develop on grafted Purkinje cells, mature parallel fibers are able to sprout, developing new terminal branches and synapses to fill the available space [103]. Indeed, the synthesis of BDNF by mature granule cells will facilitate spine production on the maturing Purkinje cell dendritic tree [104], which expresses TrkB receptors [45], suggesting that additional trophic support (particularly BDNF) is not required. However, the mature cerebellar cortex extensively expresses truncated TrkB receptors [105], which negatively regulate BDNF function [106], and additional BDNF is likely to promote parallel fiber-Purkinje cell connectivity.

In contrast, climbing fibers of the mature cerebellar cortex are much less plastic than parallel fibers. While they are capable of sprouting within the molecular layer to reinnervate adjacent denervated Purkinje cells, the overall effect is local and is limited within zebrin bands and up to a maximum of 100 μ m [107, 108], which is likely to impact on the innervation of grafted Purkinje cells. Such limited extension of climbing fiber axons has been interpreted as showing a limit to the plastic capacity of

inferior olive neurons [108], which is entirely consistent with the loss of growth factor synthesis in the adult inferior olive [90]. The hypothesis is supported by studies showing that trophic factors such as IGF-1 and BDNF can dramatically increase the growth of climbing fiber axons [109, 110] so that they develop transcommissural axon collaterals which grow through the cerebellar white matter to reinnervate both cortical Purkinje cells and DCN neurons in the contralateral hemocerebellum [111]. Not only does this innervation generate functional synapses, but it also confers recovery of lost motor and spatial learning [111]. These studies indicate that treatment with extrinsic growth factors may be a promising adjunct to Purkinje cell grafting, which facilitates the integration of the grafted cells into the existing cerebellar cortical circuit.

Inevitably, the consideration of applying neurotrophin treatment with cell grafts to human cerebellar pathology needs careful investigation of potential pitfalls. The entire process of interneuronal connectivity involves more than just the formation of new synapses but includes selective stabilization and elimination of extraneous contacts. These processes of maturation will need to be observed in host-graft interactions to ensure that new circuits are correct and will not be functionally maladaptive. This is particularly true of climbing fibers and their reinnervation of Purkinje cells, to which BDNF-TrkB signaling contributes [112]. Exogenous BDNF induces mature climbing fibers to mono-reinnervate mature Purkinje cells, but also to multiply-reinnervate immature Purkinje cells [113]. For grafted immature or progenitor Purkinje cells, such multi-innervation would need to regress for correct adult function [114]. One of the factors controlling this process of synapse maturation/elimination is retrograde Purkinje cell-climbing fiber BDNF-TrkB signaling [115, 116]. Thus, the timing of trophic factor, in particular BDNF, adjunct treatment clearly has to be highly specific to allow synapse formation, without also impacting on circuit maturation.

Clinical Implications from Neurotransplantation in Other Neurological Diseases (H. Mitoma and M. Manto)

There is a lack of information on cerebellar neurotransplantation, relative to the data on the same topic in PD [117] and Huntington's disease (HD) [9]. In PD, the dopaminergic graft is transplanted into the striatum for delivery of dopamine, whereas in HD, the aim of neurotransplantation is to replace the progressive loss of GABAergic medium spiny neurons in the striatum circuitry. The main sources of graft neurons are hESCs, hiPSCs, and neural stem cells. Characteristically, human fetal mesencephalic cells are used for PD while human fetal striatal tissues are used for HD.

Neurotransplantation has been applied more commonly in PD than in HD, since the delivery of neurotransmitters is easier than the reconstruction of striatum neural circuits. Long-term studies of PD transplantation in the last three decades have shown that the transplanted dopamine-producing cells supply sufficient amounts of dopamine, leading to movement-related activation [118, 119]. Withdrawal of levodopa (L-DOPA) is possible in most successful cases [120]. On the other hand, improvement of HD clinical symptoms has only just started to be reported.

AQ3

The above experience in transplantation in PD and HD can provide a general overview of the pros and cons of neurotransplantation and help indirectly in estimating the potential rate of therapeutic efficacy/negative effects of cerebellar neurotransplantation. The factors that influence the prognosis of PD neurotransplantation, both positively and negatively, are based on a large number of cases of neurotransplantation, as discussed below.

Prognostic Factors The outcome of neurotransplantation varies widely and is dependent on the clinical status, suggesting the existence of prognostic factors. The response to such treatment was poor in elderly patients [121] and patients with advanced disease stage [122]. It is likely that these factors are related to the paucity of synaptic plasticity and the wide range of lesions that cannot be entirely compensated by newly grafted dopaminergic neurons [123].

Negative Effects Past experience with neurotransplantation in PD suggests two types of potential negative effects: (1) side effects induced by transplantation of highly proliferative and non-self cells and (2) side effects induced by mutual actions between graft and surrounding neural circuitries.

Transplantation of highly proliferative and non-self cells can elicit carcinogenesis and immune-mediated resistance to grafts. The hESCs have high proliferative capacity and therefore can grow rapidly to form a tumor-like structure composed of neuroepithelial cells [124]. Furthermore, undifferentiated ESCs, which are difficult to harvest, can grow to a teratoma [125]. Several methods to avoid the formation of a teratoma have been described, such as fluorescence-activated cell sorting [126], induction of selective cell death using the ceramide analog *N*-oleoyl serinol [127], and manipulation of Cripto, one of the signaling molecules for the formation of a teratoma [128]. The hiPSC technology is a promising source of transplantable cells. However, since reprogramming factors of Myc and Klf4 are oncogene factors, care should be taken to protect against tumorigenesis [129]. On the other hand, the dopamine delivery function of grafted cells can be disrupted by autoimmune responses. The immune privilege of the brain is currently being reconsidered [130]. In this regard, the use of various combinations of immunosuppressants for more than 6 months after neurotransplantation has been described in many published open-label clinical trials [131].

In addition to the above potential adverse effects of neurotransplantation in PD, any interaction between graft and host neural cells can elicit unexpected neural functions and propagation of the disease. For example, recurrence of dyskinesia is reported in 15% of graft recipients within the first year [121]. Furthermore, Olanow et al. [122] reported that 65% of patients developed dyskinesia that persisted after overnight withdrawal of dopaminergic medications. Pre-existing hypersensitivity in host dopaminergic receptors, which is induced by intermittent application of L-DOPA, can exaggerate the response to dopamine released from the reinnervating dopamine graft, causing the development of dyskinesias [132]. On the other hand, Lewy bodies were observed in neurons grafted more than 10 years earlier, suggesting that α -synuclein propagates as a prion protein [133, 134]. The disease propagation process can reduce the overall benefits of neurotransplantation over the long term.

Alternative Approaches (H. Mitoma and M. Manto)

In most cerebellar ataxias (CAs), neurotransplantation is not and cannot be the sole envisioned therapy. Neurotransplantation is an invasive procedure, and potential serious adverse events have to be taken into account (see paragraph 6). Furthermore, there is still no clinical evidence for its effectiveness in CAs. Overall, the application of neurotransplantation is based on two cellular and functional concepts: cell rescue and facilitation of cerebellar reserve. Both could be potentially achieved also with other approaches. The third concept, cell substitution, is an exclusive task for neurotransplantation, if not taking into account theoretical possibility of sufficient activation of endogenous neurogenesis.

Cell Rescue

In case of “progressive pathologies,” the available therapies are designed to prevent further damage and rescue the cells from degeneration [6, 41, 135] and are therefore closely related to the pathogenesis of each particular disease. For instance, in metabolic- and immune-mediated CAs, treatment is based on rectification of the causal factor—metabolic disorder or autoimmune process, respectively. Thus, these CAs are mostly *controllable*. On the other hand, in hereditary neurodegenerative CAs, there is no effective treatment, so potential therapies are under development and their *controllability* is current rather than hypothetical. Molecular-based therapy should be a first option. Experiments in mouse models of SCAs showed some prospective possibilities even in degenerative SCAs [136, 137, 138, 139, 140, 141, 142, 143].

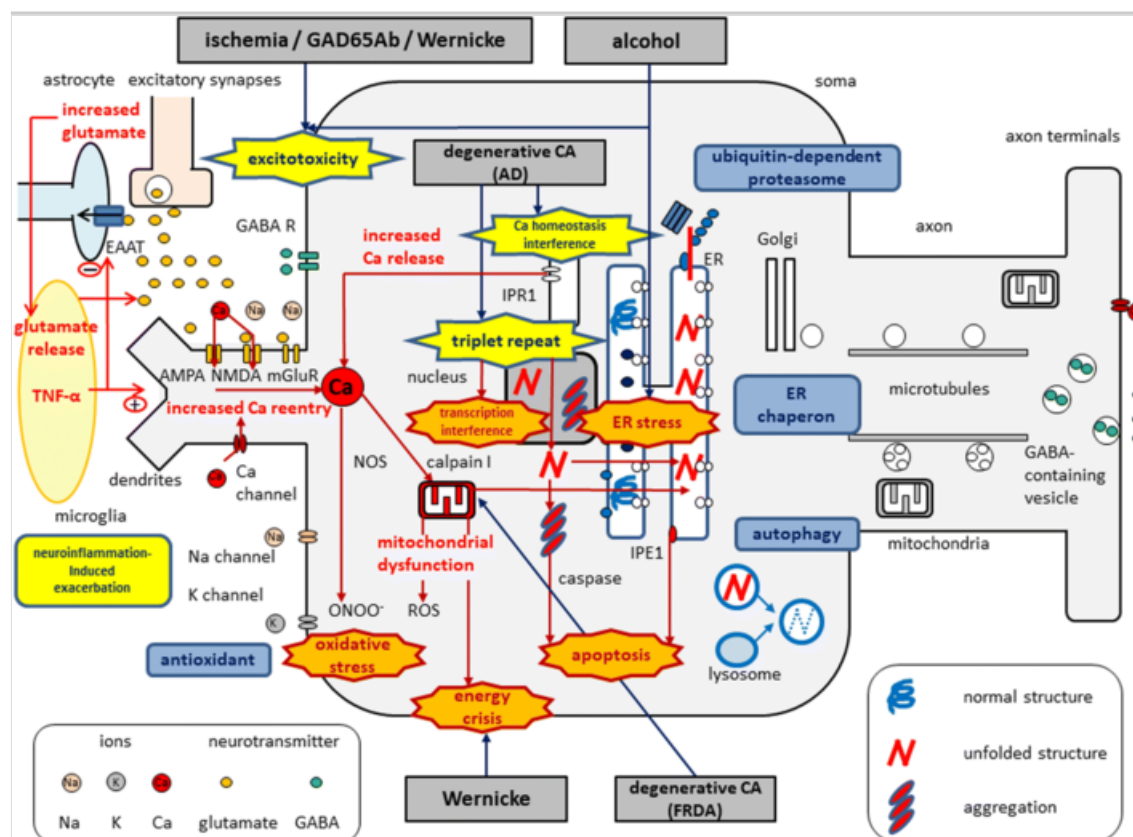
AQ4

Targeting the RNA is a promising therapeutic approach in degenerative diseases of the cerebellum, through the down- or upregulation of the expression of the target pathogenic gene [144]. In particular,

methods that use RNA interference (RNAi) and bioactive small molecules can act as suppressors of a particular gene, whereas intrathecal delivery technology of mRNA encapsulated in lipid nanoparticles can upregulate the expression of a given gene. In animal experiments, molecular-targeted therapy has been reported to halt disease progression and rescue cells from cell death [136, 137, 138, 139, 140, 141, 142, 143]. For example, accelerating cleavage of the pathological protein containing extended polyglutamine tract is effective in SCA3 and SCA7 mice [140, 143]. Treatment with dantrolene, a calcium ion stabilizer, in SCA2 and SCA3 mice is associated with improved motor performance and prevention of neuronal cell loss in both the pontine nuclei and substantia nigra [136, 138]. Although there are diverse causes and mechanisms of impaired cerebellar circuitry, the main invasive actions converge on common pathological elements such as ER stress, oxidative stress, energy crisis, dysregulation of transcription and gene expression, and apoptosis (Fig. 3). Where common final pathways are shared between etiologies (for example, ER stress in anti-GAD ataxia and ethanol-induced cerebellar atrophy), molecular-based therapies identified for these processes (ER stress in this case) could also have therapeutic benefit in patients with immune-mediated or metabolic CAs who showed resistance to immune- or metabolic-mediated therapies, respectively.

Fig. 3

A schematic diagram of main pathways leading to cerebellar Purkinje cell death. Although there are diverse etiologies (indicated by gray boxes) which impair the cerebellum, main invasive reactions converge on several common pathological elements (indicated by yellow boxes): (1) triplet repeats in genes eliciting a production of unfolded proteins and aggregation, (2) interference in calcium homeostasis, and (3) excitotoxicity causing increased calcium entry. The extracellular invasions, excitotoxicity, are characterized by enhancement through microglial cell-induced neuroinflammation with a positive-feedback fashion. These pathological elements, in turn, form chained cascades (indicated by red fonts), leading to the lethal status of the cells (indicated by orange boxes): (1) ER stress, (2) oxidative stress, (3) energy crisis, (4) dysregulation of transcription and gene expression, and (5) apoptosis. The status can be chained into following core cascades: (1) triplet repeats → pathological protein aggregates → interference with transcription, apoptosis, and ER stress; (2) excessive calcium → mitochondrial dysfunction and oxidative stress; and (3) mitochondrial dysfunction → ER stress → apoptosis. Importantly, these cell stresses are offset by (1) ER chaperon, ubiquitin-dependent proteasome, and autophagy and (2) antioxidant agents (indicated by blue boxes). Thus, the lethal status, which is beyond these protective capacities, elicits cell death. These protective capacities consist of cellular reserve and cellular abilities for compensation and restoration. Theoretically, molecular-based or neurotransplantation therapies could work via reduction of the pathological elements and interference with the cascades, leading to lethal status or via supporting protective capacities



However, both the efficacy and safety of such therapy have not been verified yet. Furthermore, the clinical condition can also have certain peculiarities that hinder the effectiveness of such therapy. For example, in the case of hereditary degenerative CAs, deleterious effects of abnormal proteins persist despite treatment, and thus, the need for alternative permanent therapy would be necessary to prevent cell death. Moreover, the safety of repeated molecular manipulation in patients is uncertain. Another problem related to molecular-based therapy is that it is strictly specific for individual subtypes of disease based on the different underlying pathogenic processes involved in cell degeneration (Fig. 3). Therapies must be established for each subtype within the CAs, so delays are expected.

Significant advantages have not yet been gained from molecular-based therapies. The same comment is valid for neurotransplantation. It is also unclear whether synergistic effects will occur or not. Importantly, although both molecular-based therapies and neurotransplantation could act on the lethal status of specific cell pathologies and prevent/delay cell death, detailed molecular mechanisms involved remain unclear.

Facilitation on Cerebellar Reserve

In cases of “acute (unexpected) pathologies” with irreversible tissue damage, such as stroke or injury, and for conditions of “progressive pathologies” including “potentially controllable” and “uncontrollable,” therapies are expected to support residual cerebellar functions [135]. While cerebellar reserve defined as the capacity of the neural tissue for functional restoration and compensation to pathology [41] is preserved, appropriate therapies can facilitate long-term improvement. Examples of the established neuromodulation therapies, which facilitate cerebellar reserve, include motor rehabilitation and non-invasive cerebellar stimulation. The first option is intensive motor rehabilitation which improves motor functions [145]. The second, non-invasive cerebellar stimulation, including repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), is promising. Indeed, non-invasive cerebellar stimulation promotes synaptic plasticity and, therefore, has great potential for the reconstruction of lost cerebellar functions [146]. In fact, the therapeutic benefits of tDCS were reported in a double-blind, randomized, sham-controlled study [147], in which a 2-week treatment with cerebellar anodal tDCS improved ataxia rating scores (scale for the assessment and rating

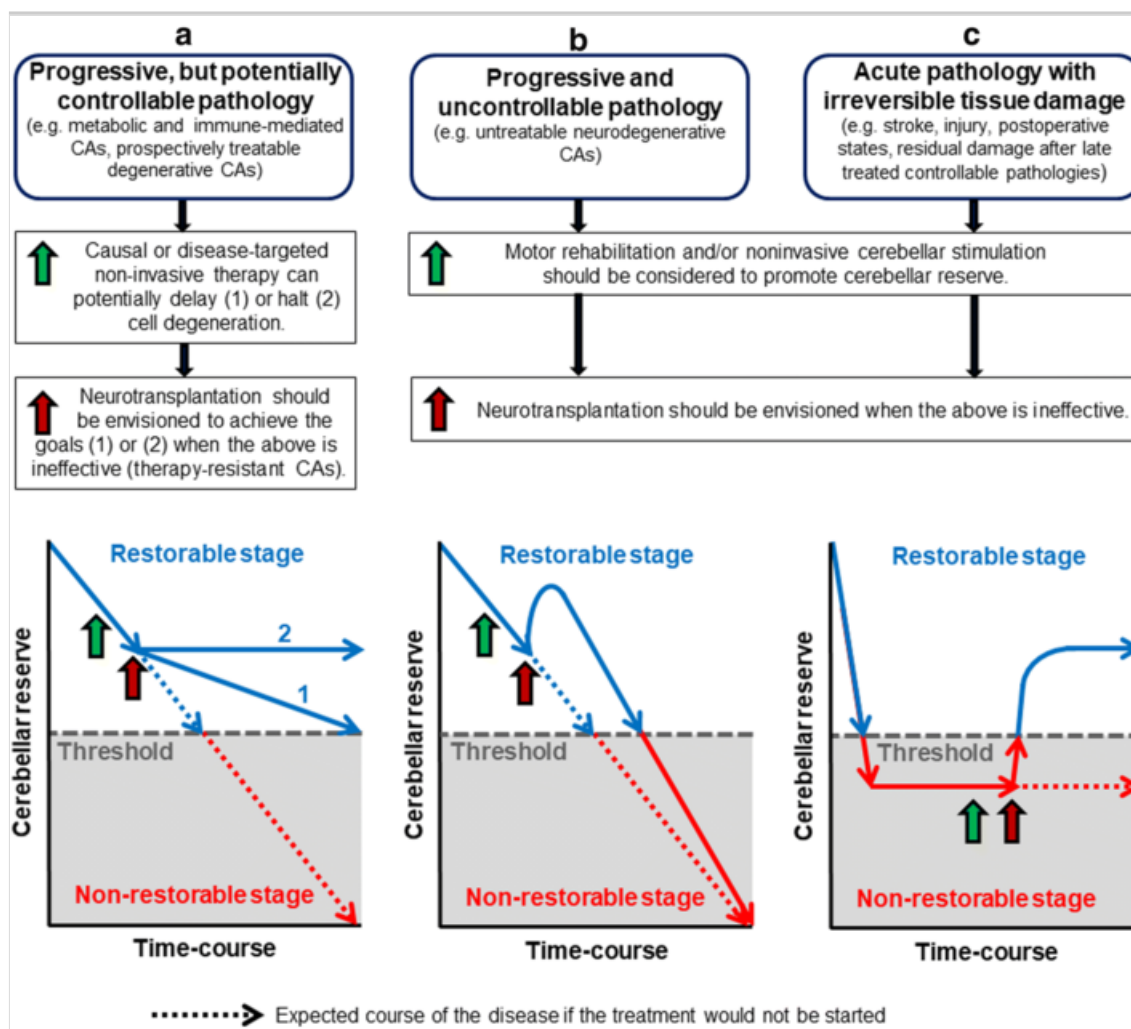
of ataxia SARA, International Cooperative Ataxia Rating Scale (ICARS), 9-hole peg test, 8 m walking time) and cerebellar brain inhibition (CBI) [148].

As long as improvement gained through these neuromodulation therapies does not reach the critical level of motor function necessary for the daily activities of the patient, neurotransplantation remains an option to be considered. However, one difficulty is that the community still has an unclear picture of the short- and long-term effects of motor rehabilitation/non-invasive cerebellar stimulation and their biological mechanisms. Neurotransplantation should be applied after exhaustive trials of the neuromodulation therapies in *acute pathologies* and *progressive pathologies*. The interactions between drugs and neuromodulation therapies are also unknown.

Lastly, it has to be remembered that cerebellar disorders comprise a myriad of diseases. Based on the clinical time course of each disease process, the superiority or inferiority of each therapy should be considered before the selection of neurotransplantation. A first treatment algorithm is summarized in Fig. 4.

Fig. 4

A flow diagram of the recommended algorithm for selection of cerebellar neurotransplantation. The bottom figures show the course of the disease and the potential influence of the therapy. As the disease progresses, cerebellar reserve decreases. (A) *Cell rescue*. In case of progressive but potentially controllable pathology, the aim of neurotransplantation is to slow the progression of the disease process (change of the slope of cerebellar reserve decline) by cell rescue. The therapy should be started in early stages of the disease before the cerebellum falls into the non-restorable state. (B, C) *Facilitation on cerebellar reserve*. In the case of progressive and uncontrollable pathology (B) and acute pathology with irreversible tissue damage (C), cell loss cannot be prevented. The purpose of neurotransplantation is to facilitate cerebellar reserve (upward shift). In the case of progressive pathology (B), neurotransplantation should be conducted while cerebellar reserve is preserved. Decline of cerebellar function continues but due to potentiation of cerebellar reserve, the non-restorable state comes later. In the case of irreversible cell loss (C), cell substitution might potentially replenish cerebellar reserve and turn the cerebellum into restorable stage. The upward arrow in each figure indicates the conduct of neurotransplantation (red arrow) and/or alternative therapy (green arrow). The figures show the case that alternative therapy is not effective (now change of curve) and, thus, neurotransplantation is used



Conclusion (J. Cendelin and F. Vozech)

Neurotransplantation is one of potential future therapies for cerebellar pathologies. Nevertheless, there are still many doubts and unknown aspects that evoke the necessity of careful approach, always respecting the safety of the patients. Most of our knowledge about cerebellar transplantation, both optimistic and pessimistic, comes from experiments in animals, namely mice, and to date, there are only few data from human patients. Animal models have their limitations, and translation of the findings to human medicine is not easy. Some information can be extrapolated from neurotransplantation therapy for other diseases, such as PD and HD. Nevertheless, these diseases have their own specifics different from cerebellar disorders. Thus, there are only indirect indices that neurotransplantation could also be applicable for the cerebellum in humans. Adequate direct evidence is still lacking.

The cerebellum belongs among the non-neurogenic structures which limits its self-recovery capacity and might also hinder graft survival and integration. Furthermore, diverse cerebellar functions are strongly related to its complex microstructure consisting of myriads of many types of cells connected through precise point-to-point circuits that are difficult to reconstruct by grafted cells. Cerebellar diseases, particularly cerebellar degeneration, are a heterogeneous group of pathologies with various pathogenic processes and various combinations of cerebellar and extracerebellar damage and are accompanied with various changes of the cerebellar tissue niche. For all of that, transplantation therapy for cerebellar diseases is expected to be more problematic than that for, e.g., PD. It is possible that it will not bring the same effect in all types of cerebellar diseases and that we will need disease-specific approach.

Biological aspects and limitations of neurotransplantation were discussed here as well as elsewhere [1, 2, 3, 4, 6, 135]. Nevertheless, there are also ethical aspects concerning, in particular, embryonic stem cells and embryonic or fetal neural progenitors. Advancement in technology of in vitro propagation and

cellular differentiation makes available new types of cells which are available in larger quantities and without the associated ethical problems.

Thanks to the development of other molecular approaches, pharmacotherapy of neurodegenerative diseases or neurostimulation methods might become effective in the treatment of many brain, including cerebellar, diseases so that transplantation will not be needed in many cases. Nevertheless, it is too early to draw conclusions in this regard.

Anyway, neurotransplantation research is not only important for the development of neurotransplantation therapy as a clinical tool of patient treatment but helps also understand brain development, rules of stem cell proliferation, cellular migration and differentiation, or self-regenerative processes and mechanisms of brain plasticity. Indeed, searching for tools for neurotransplantation therapy accelerated stem cell research and increased our knowledge of cerebellar development and factors influencing cell survival, differentiation, migration, etc. If nothing else, this is a significant contribution to biomedical sciences.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors' Contribution

The authors were responsible for drafting specific sections, and all of them revised and contributed to the entire article.

Funding Information

AQ5

This work was supported by the National Sustainability Program I (NPU I) (no. LO1503) provided by the Ministry of Education, Youth and Sports of the Czech Republic; by the Charles University Research Fund (project number Q39), MEXT KAKENHI (grant number 15H04254); and by the Gunma University Initiative for Advanced Research (GIAR), Funds of the University of Turin and of the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) project "Dipartimenti di Eccellenza 2018–2022" to the Department of Neuroscience, University of Turin.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Rossi F, Cattaneo E. Opinion: neural stem cell therapy for neurological diseases: dreams and reality. *Nat Rev Neurosci*. 2002;3:401–9.
2. Carletti B, Piemonte F, Rossi F. Neuroprotection: the emerging concept of restorative neural stem cell biology for the treatment of neurodegenerative diseases. *Curr Neuropharmacol*. 2011;9:313–7.
3. Cendelin J. Experimental neurotransplantation treatment for hereditary cerebellar ataxias. *Cerebellum Ataxias*. 2016a;3:7.
4. Cendelin J. Transplantation and stem cell therapy for cerebellar degenerations. *Cerebellum*. 2016b;15:48–50.
5. Kumar A, Narayanan K, Chaudhary RK, Mishra S, Kumar S, Vinoth KJ, et al. Current perspective of stem cell therapy in neurodegenerative and metabolic diseases. *Mol Neurobiol*. 2017;54(9):7276–

96.

6. Cendelin J, Mitoma H, Manto M. Neurotransplantation therapy and cerebellar reserve. *CNS Neurol Disord Drug Targets*. 2018a;17(3):172–83.

7. Grade S, Gotz M. Neuronal replacement therapy: previous achievements and challenges ahead. *NPJ Regener Med*. 2017;2:29.

8. Parmar M. Towards stem cell based therapies for Parkinson's disease. *Development*. 2018;145.

AQ6

9. Barker RA, Barrett J, Mason SL, Bjorklund A. Fetal dopaminergic transplantation trials and the future of neural grafting in Parkinson's disease. *Lancet Neurol*. 2013;12:84–91.

10. Barker RA, Studer L, Cattaneo E, Takahashi J. G-Force PD: a global initiative in coordinating stem cell-based dopamine treatments for Parkinson's disease. *NPJ Park Dis*. 2015;1:15017.

11. Sotelo C, Alvarado-Mallart RM. Embryonic and adult neurons interact to allow Purkinje cell replacement in mutant cerebellum. *Nature*. 1987;327:421–3.

12. Triarhou LC, Low WC, Ghatti B. Transplantation of cerebellar anlagen to hosts with genetic cerebellocortical atrophy. *Anat Embryol*. 1987;176:145–54.

13. Kohsaka S, Takayama H, Ueda T, Toya S, Tsukada Y. Reorganization of cerebellar cell suspension transplanted into the weaver mutant cerebellum and immunohistochemical detection of synaptic formation. *Neurosci Res*. 1988;6:162–6.

14. Dumesnil-Bousez N, Sotelo C. Partial reconstruction of the adult Lurcher cerebellar circuitry by neural grafting. *Neuroscience*. 1993;55:1–21.

15. Tomey DA, Heckroth JA. Transplantation of normal embryonic cerebellar cell suspensions into the cerebellum of lurcher mutant mice. *Exp Neurol*. 1993;122:165–70.

16. Heckroth JA, Hobart NJ, Summers D. Transplanted neurons alter the course of neurodegenerative disease in Lurcher mutant mice. *Exp Neurol*. 1998;154:336–52.

17. Cendelin J, Korelusova I, Vozeh F. A preliminary study of solid embryonic cerebellar graft survival in adult B6CBA Lurcher mutant and wild type mice. *Anat Rec (Hoboken)*. 2009;292:1986–92.

18. Cendelin J, Babuska V, Korelusova I, Houdek Z, Vozeh F. Long-term survival of solid embryonic cerebellar grafts in Lurcher mice. *Neurosci Lett*. 2012;515:23–7.

19. Purkartova Z, Tuma J, Pesta M, Kulda V, Hajkova L, Sebesta O, et al. Morphological analysis of embryonic cerebellar grafts in SCA2 mice. *Neurosci Lett*. 2014;558:154–8.

20. Cendelin J, Purkartova Z, Kubik J, Ulbricht E, Tichanek F, Kolinko Y. Long-term development of embryonic cerebellar grafts in two strains of lurcher mice. *Cerebellum*. 2018b;17(4):428–37.

21. Li J, Imitola J, Snyder EY, Sidman RL. Neural stem cells rescue nervous Purkinje neurons by restoring molecular homeostasis of tissue plasminogen activator and downstream targets. *J Neurosci*. 2006;26:7839–48.

22. Roybon L, Ma Z, Asztely F, Fosum A, Jacobsen SE, Brundin P, et al. Failure of transdifferentiation of adult hematopoietic stem cells into neurons. *Stem Cells*. 2006;24:1594–604.
23. Sidman RL, Li J, Stewart GR, Clarke J, Yang W, Snyder EY, et al. Injection of mouse and human neural stem cells into neonatal Niemann-Pick A model mice. *Brain Res*. 2007;1140:195–204.
24. Chen KA, Lanuto D, Zheng T, Steindler DA. Transplantation of embryonic and adult neural stem cells in the granulo-prival cerebellum of the weaver mutant mouse. *Stem Cells*. 2009;27:1625–34.
25. Chintawar S, Hourez R, Ravella A, Gall D, Orduz D, Rai M, et al. Grafting neural precursor cells promotes functional recovery in an SCA1 mouse model. *J Neurosci*. 2009;29:13126–35.
26. Jaderstad J, Jaderstad LM, Li J, Chintawar S, Salto C, Pandolfo M, et al. Communication via gap junctions underlies early functional and beneficial interactions between grafted neural stem cells and the host. *Proc Natl Acad Sci U S A*. 2010;107:5184–9.
27. Takayama H, Kohsaka S, Shinozaki T, Inoue H, Toya S, Ueda T, et al. Immunohistochemical studies on synapse formation by embryonic cerebellar tissue transplanted into the cerebellum of the weaver mutant mouse. *Neurosci Lett*. 1987;79:246–50.
28. Takayama H, Toya S, Shinozaki T, Inoue H, Otani M, Kohsaka S, et al. Possible synapse formation by embryonic cerebellar tissue grafted into the cerebellum of the weaver mutant mouse. *Acta Neurochir Suppl*. 1988;43:154–8.
29. Gardette R, Alvarado-Mallart RM, Crepel F, Sotelo C. Electrophysiological demonstration of a synaptic integration of transplanted Purkinje cells into the cerebellum of the adult Purkinje cell degeneration mutant mouse. *Neuroscience*. 1988;24:777–89.
30. Sotelo C, Alvarado-Mallart RM. The reconstruction of cerebellar circuits. *Trends Neurosci*. 1991;14:350–5.
31. Triarhou LC, Zhang W, Lee WH. Graft-induced restoration of function in hereditary cerebellar ataxia. *Neuroreport*. 1995;6:1827–32.
32. Triarhou LC, Zhang W, Lee WH. Amelioration of the behavioral phenotype in genetically ataxic mice through bilateral intracerebellar grafting of fetal Purkinje cells. *Cell Transplant*. 1996;5:269–77.
33. Kaemmerer WF, Low WC. Cerebellar allografts survive and transiently alleviate ataxia in a transgenic model of spinocerebellar ataxia type-1. *Exp Neurol*. 1999;158:301–11.
34. Babuska V, Houdek Z, Tuma J, Purkartova Z, Tumova J, Kralickova M, et al. Transplantation of embryonic cerebellar grafts improves gait parameters in ataxic lurcher mice. *Cerebellum*. 2015;14:632–41.
35. Fuca E, Guglielmotto M, Boda E, Rossi F, Leto K, Buffo A. Preventive motor training but not progenitor grafting ameliorates cerebellar ataxia and deregulated autophagy in tambaleante mice. *Neurobiol Dis*. 2017;102:49–59.
36. Bae JS, Furuya S, Ahn SJ, Yi SJ, Hirabayashi Y, Jin HK. Neuroglial activation in Niemann-Pick type C mice is suppressed by intracerebral transplantation of bone marrow-derived mesenchymal stem cells. *Neurosci Lett*. 2005;381:234–6.

37. Jones J, Jaramillo-Merchan J, Bueno C, Pastor D, Viso-Leon M, Martinez S. Mesenchymal stem cells rescue Purkinje cells and improve motor functions in a mouse model of cerebellar ataxia. *Neurobiol Dis.* 2010;40:415–23.
38. Lee H, Lee JK, Min WK, Bae JH, He X, Schuchman EH, et al. Bone marrow-derived mesenchymal stem cells prevent the loss of Niemann-Pick type C mouse Purkinje neurons by correcting sphingolipid metabolism and increasing sphingosine-1-phosphate. *Stem Cells.* 2010;28:821–31.
39. Matsuura S, Shuvaev AN, Iizuka A, Nakamura K, Hirai H. Mesenchymal stem cells ameliorate cerebellar pathology in a mouse model of spinocerebellar ataxia type 1. *Cerebellum.* 2014;13:323–30.
40. Mendonca LS, Nobrega C, Hirai H, Kaspar BK, Pereira de Almeida L. Transplantation of cerebellar neural stem cells improves motor coordination and neuropathology in Machado-Joseph disease mice. *Brain.* 2015;138:320–35.
41. Mitoma H, Manto M. The physiological basis of therapies for cerebellar ataxias. *Ther Adv Neurol Disord.* 2016;9:396–413.
42. Bae JS, Carter JE, Jin HK. Adipose tissue-derived stem cells rescue Purkinje neurons and alleviate inflammatory responses in Niemann-Pick disease type C mice. *Cell Tissue Res.* 2010;340:357–69.
43. Martins LF, Costa RO, Pedro JR, Aguiar P, Serra SC, Teixeira FG, et al. Mesenchymal stem cells secretome-induced axonal outgrowth is mediated by BDNF. *Sci Rep.* 2017;7:4153.
44. Reidling JC, Relano-Gines A, Holley SM, Ochaba J, Moore C, Fury B, et al. Human neural stem cell transplantation rescues functional deficits in R6/2 and Q140 Huntington's disease mice. *Stem Cell Rep.* 2018;10:58–72.
45. Carter AR, Chen C, Schwartz PM, Segal RA. Brain-derived neurotrophic factor modulates cerebellar plasticity and synaptic ultrastructure. *J Neurosci.* 2002;22:1316–27.
46. Huang Y, Ko H, Cheung ZH, Yung KK, Yao T, Wang JJ, et al. Dual actions of brain-derived neurotrophic factor on GABAergic transmission in cerebellar Purkinje neurons. *Exp Neurol.* 2012;233:791–8.
47. Watson LM, Wong MM, Becker EB. Induced pluripotent stem cell technology for modelling and therapy of cerebellar ataxia. *Open Biol.* 2015;5:150056.
48. Wong MMK, Watson LM, Becker EBE. Recent advances in modelling of cerebellar ataxia using induced pluripotent stem cells. *J Neurol Neuromedicine.* 2017;2:11–5.
49. Su HL, Muguruma K, Matsuo-Takasaki M, Kengaku M, Watanabe K, Sasai Y. Generation of cerebellar neuron precursors from embryonic stem cells. *Dev Biol.* 2006;290:287–96.
50. Salero E, Hatten ME. Differentiation of ES cells into cerebellar neurons. *Proc Natl Acad Sci U S A.* 2007;104:2997–3002.
51. Tao O, Shimazaki T, Okada Y, Naka H, Kohda K, Yuzaki M, et al. Efficient generation of mature cerebellar Purkinje cells from mouse embryonic stem cells. *J Neurosci Res.* 2010;88:234–47.

52. Muguruma K, Nishiyama A, Ono Y, Miyawaki H, Mizuhara E, Hori S, et al. Ontogeny-recapitulating generation and tissue integration of ES cell-derived Purkinje cells. *Nat Neurosci.* 2010;13:1171–80.
53. Higuera GA, Iaffaldano G, Bedar M, Shpak G, Broersen R, Munshi ST, et al. An expandable embryonic stem cell-derived Purkinje neuron progenitor population that exhibits in vivo maturation in the adult mouse cerebellum. *Sci Rep.* 2017;7:8863.
54. Wang S, Wang B, Pan N, Fu L, Wang C, Song G, et al. Differentiation of human induced pluripotent stem cells to mature functional Purkinje neurons. *Sci Rep.* 2015;5:9232.
55. Ishida Y, Kawakami H, Kitajima H, Nishiyama A, Sasai Y, Inoue H, et al. Vulnerability of Purkinje cells generated from spinocerebellar ataxia type 6 patient-derived iPSCs. *Cell Rep.* 2016;17:1482–90.
56. Sundberg M, Tochitsky I, Buchholz DE, Winden K, Kujala V, Kapur K, et al. Purkinje cells derived from TSC patients display hypoexcitability and synaptic deficits associated with reduced FMRP levels and reversed by rapamycin. *Mol Psychiatry.* 2018;23:2167–83.
57. Muguruma K, Nishiyama A, Kawakami H, Hashimoto K, Sasai Y. Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. *Cell Rep.* 2015;10:537–50.
58. Watson LM, Wong MMK, Vowles J, Cowley SA, Becker EBE. A simplified method for generating Purkinje cells from human-induced pluripotent stem cells. *Cerebellum.* 2018;17:419–27.
59. Carletti B, Grimaldi P, Magrassi L, Rossi F. Specification of cerebellar progenitors after heterotopic-heterochronic transplantation to the embryonic CNS in vivo and in vitro. *J Neurosci.* 2002;22:7132–46.
60. Zhang W, Lee WH, Triarhou LC. Grafted cerebellar cells in a mouse model of hereditary ataxia express IGF-I system genes and partially restore behavioral function. *Nat Med.* 1996;2:65–71.
61. Magrassi L, Leto K, Rossi F. Lifespan of neurons is uncoupled from organismal lifespan. *Proc Natl Acad Sci U S A.* 2013;110:4374–9.
62. Carletti B, Williams IM, Leto K, Nakajima K, Magrassi L, Rossi F. Time constraints and positional cues in the developing cerebellum regulate Purkinje cell placement in the cortical architecture. *Dev Biol.* 2008;317:147–60.
63. Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z, et al. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature.* 2011;480:547–51.
64. Grealish S, Diguët E, Kirkeby A, Mattsson B, Heuer A, Bramoulle Y, et al. Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. *Cell Stem Cells.* 2014;15:653–65.
65. Michelsen KA, Acosta-Verdugo S, Benoit-Marand M, Espuny-Camacho I, Gaspard N, Saha B, et al. Area-specific reestablishment of damaged circuits in the adult cerebral cortex by cortical neurons derived from mouse embryonic stem cells. *Neuron.* 2015;85:982–97.

66. Steinbeck JA, Choi SJ, Mrejeru A, Ganat Y, Deisseroth K, Sulzer D, et al. Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson's disease model. *Nat Biotechnol.* 2015;33:204–9.
67. Falkner S, Grade S, Dimou L, Conzelmann KK, Bonhoeffer T, Gotz M, et al. Transplanted embryonic neurons integrate into adult neocortical circuits. *Nature.* 2016;539:248–53.
68. Faedo A, Laporta A, Segnali A, Galimberti M, Besusso D, Cesana E, et al. Differentiation of human telencephalic progenitor cells into MSNs by inducible expression of Gsx2 and Ebf1. *Proc Natl Acad Sci U S A.* 2017;114:E1234–e42.
69. Kikuchi T, Morizane A, Doi D, Magotani H, Onoe H, Hayashi T, et al. Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. *Nature.* 2017;548:592–6.
70. Sotelo C, Alvarado-Mallart RM, Frain M, Vernet M. Molecular plasticity of adult Bergmann fibers is associated with radial migration of grafted Purkinje cells. *J Neurosci.* 1994;14:124–33.
71. Miyata T, Nakajima K, Aruga J, Takahashi S, Ikenaka K, Mikoshiba K, et al. Distribution of a reeler gene-related antigen in the developing cerebellum: an immunohistochemical study with an allogeneic antibody CR-50 on normal and reeler mice. *J Comp Neurol.* 1996;372:215–28.
72. Miyata T, Nakajima K, Mikoshiba K, Ogawa M. Regulation of Purkinje cell alignment by reelin as revealed with CR-50 antibody. *J Neurosci.* 1997;17:3599–609.
73. Rosenfeld JV, Richards LJ, Bartlett PF. Mutant mouse cerebellum does not provide specific signals for the selective migration and development of transplanted Purkinje cells. *Neurosci Lett.* 1993;155:19–23.
74. de Luca A, Vassallo S, Benitez-Temino B, Menichetti G, Rossi F, Buffo A. Distinct modes of neuritic growth in Purkinje neurons at different developmental stages: axonal morphogenesis and cellular regulatory mechanisms. *PLoS One.* 2009;4:e6848.
75. Kordower JH, Goetz CG, Chu Y, Halliday GM, Nicholson DA, Musial TF, et al. Robust graft survival and normalized dopaminergic innervation do not obligate recovery in a Parkinson disease patient. *Ann Neurol.* 2017;81:46–57.
76. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med.* 1999;341:738–46.
77. Krampera M, Franchini M, Pizzolo G, Aprili G. Mesenchymal stem cells: from biology to clinical use. *Blood Transfus.* 2007;5:120–9.
78. Johnson TV, DeKorver NW, Levasseur VA, Osborne A, Tassoni A, Lorber B, et al. Identification of retinal ganglion cell neuroprotection conferred by platelet-derived growth factor through analysis of the mesenchymal stem cell secretome. *Brain.* 2014;137:503–19.
79. Yang Y, Ye Y, Su X, He J, Bai W, He X. MSCs-derived exosomes and neuroinflammation, neurogenesis and therapy of traumatic brain injury. *Front Cell Neurosci.* 2017;11:55.
80. Lo Furno D, Mannino G, Giuffrida R. Functional role of mesenchymal stem cells in the treatment of chronic neurodegenerative diseases. *J Cell Physiol.* 2018;233:3982–99.
81. Chang YK, Chen MH, Chiang YH, Chen YF, Ma WH, Tseng CY, et al. Mesenchymal stem cell transplantation ameliorates motor function deterioration of spinocerebellar ataxia by rescuing

cerebellar Purkinje cells. *J Biomed Sci.* 2011;18:54.

82. Mieda T, Suto N, Iizuka A, Matsuura S, Iizuka H, Takagishi K, et al. Mesenchymal stem cells attenuate peripheral neuronal degeneration in spinocerebellar ataxia type 1 knockin mice. *J Neurosci Res.* 2016;94:246–52.

83. Chen KA, Cruz PE, Lanuto DJ, Flotte TR, Borchelt DR, Srivastava A, et al. Cellular fusion for gene delivery to SCA1 affected Purkinje neurons. *Mol Cell Neurosci.* 2011;47:61–70.

84. Bae JS, Han HS, Youn DH, Carter JE, Modo M, Schuchman EH, et al. Bone marrow-derived mesenchymal stem cells promote neuronal networks with functional synaptic transmission after transplantation into mice with neurodegeneration. *Stem Cells.* 2007;25:1307–16.

85. Huda F, Fan Y, Suzuki M, Konno A, Matsuzaki Y, Takahashi N, et al. Fusion of human fetal mesenchymal stem cells with “degenerating” cerebellar neurons in spinocerebellar ataxia type 1 model mice. *PLoS One.* 2016;11:e0164202.

86. Weimann JM, Johansson CB, Trejo A, Blau HM. Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. *Nat Cell Biol.* 2003;5:959–66.

87. Johansson CB, Youssef S, Koleckar K, Holbrook C, Doyonnas R, Corbel SY, et al. Extensive fusion of haematopoietic cells with Purkinje neurons in response to chronic inflammation. *Nat Cell Biol.* 2008;10:575–83.

88. Magrassi L, Grimaldi P, Ibatici A, Corselli M, Ciardelli L, Castello S, et al. Induction and survival of binucleated Purkinje neurons by selective damage and aging. *J Neurosci.* 2007;27:9885–92.

89. Park HW, Chang JW, Yang YS, Oh W, Hwang JH, Kim DG, et al. The effect of donor-dependent administration of human umbilical cord blood-derived mesenchymal stem cells following focal cerebral ischemia in rats. *Exp Neurol.* 2015;24:358–65.

90. Sherrard RM, Bower AJ. Climbing fiber development: do neurotrophins have a part to play? *Cerebellum.* 2002;1:265–75.

91. Wilkins A, Kemp K, Ginty M, Hares K, Mallam E, Scolding N. Human bone marrow-derived mesenchymal stem cells secrete brain-derived neurotrophic factor which promotes neuronal survival in vitro. *Stem Cell Res.* 2009;3:63–70.

92. Dusart I, Airaksinen MS, Sotelo C. Purkinje cell survival and axonal regeneration are age dependent: an in vitro study. *J Neurosci.* 1997;17:3710–26.

93. Eisenman LM, Schalekamp MP, Voogd J. Development of the cerebellar cortical efferent projection: an in-vitro anterograde tracing study in rat brain slices. *Brain Res Dev Brain Res.* 1991;60:261–6.

94. Goffinet AM. The embryonic development of the cerebellum in normal and reeler mutant mice. *Anat Embryol.* 1983;168:73–86.

95. Sergaki MC, Ibanez CF. GFR α 1 regulates Purkinje cell migration by counteracting NCAM function. *Cell Rep.* 2017;18:367–79.

96. Kapfhammer JP. Cellular and molecular control of dendritic growth and development of cerebellar Purkinje cells. *Prog Histochem Cytochem*. 2004;39:131–82.
97. Sotelo C, Dusart I. Intrinsic versus extrinsic determinants during the development of Purkinje cell dendrites. *Neuroscience*. 2009;162:589–600.
98. Torres-Aleman I, Pons S, Arevalo MA. The insulin-like growth factor I system in the rat cerebellum: developmental regulation and role in neuronal survival and differentiation. *J Neurosci Res*. 1994;39:117–26.
99. Nieto-Bona MP, Garcia-Segura LM, Torres-Aleman I. Transynaptic modulation by insulin-like growth factor I of dendritic spines in Purkinje cells. *Int J Dev Neurosci*. 1997;15:749–54.
100. Sadakata T, Kakegawa W, Mizoguchi A, Washida M, Katoh-Semba R, Shutoh F, et al. Impaired cerebellar development and function in mice lacking CAPS2, a protein involved in neurotrophin release. *J Neurosci*. 2007;27:2472–82.
101. Borghesani PR, Peyrin JM, Klein R, Rubin J, Carter AR, Schwartz PM, et al. BDNF stimulates migration of cerebellar granule cells. *Development*. 2002;129:1435–42.
102. Tsutsui K, Ukena K, Sakamoto H, Okuyama S, Haraguchi S. Biosynthesis, mode of action, and functional significance of neurosteroids in the Purkinje cell. *Front Endocrinol*. 2011;2:61.
103. Chen S, Hillman DE. Marked reorganization of Purkinje cell dendrites and spines in adult rat following vacating of synapses due to deafferentation. *Brain Res*. 1982;245:131–5.
104. Shimada A, Mason CA, Morrison ME. TrkB signaling modulates spine density and morphology independent of dendrite structure in cultured neonatal Purkinje cells. *J Neurosci*. 1998;18:8559–70.
105. Ohira K, Funatsu N, Nakamura S, Hayashi M. Expression of BDNF and TrkB receptor subtypes in the postnatal developing Purkinje cells of monkey cerebellum. *Gene Expr Patterns*. 2004;4:257–61.
106. Lei L, Parada LF. Transcriptional regulation of Trk family neurotrophin receptors. *Cell Mol Life Sci*. 2007;64:522–32.
107. Rossi F, Wiklund L, van der Want JJ, Strata P. Reinnervation of cerebellar Purkinje cells by climbing fibres surviving a subtotal lesion of the inferior olive in the adult rat. I. Development of new collateral branches and terminal plexuses. *J Comp Neurol*. 1991;308:513–35.
108. Dhar M, Brenner JM, Sakimura K, Kano M, Nishiyama H. Spatiotemporal dynamics of lesion-induced axonal sprouting and its relation to functional architecture of the cerebellum. *Nat Commun*. 2016;7:12938.
109. Dixon KJ, Sherrard RM. Brain-derived neurotrophic factor induces post-lesion transcommissural growth of olivary axons that develop normal climbing fibers on mature Purkinje cells. *Exp Neurol*. 2006;202:44–56.
110. Sherrard RM, Bower AJ. IGF-1 induces neonatal climbing-fibre plasticity in the mature rat cerebellum. *Neuroreport*. 2003;14:1713–6.
111. Willson ML, McElnea C, Mariani J, Lohof AM, Sherrard RM. BDNF increases homotypic olivocerebellar reinnervation and associated fine motor and cognitive skill. *Brain*. 2008;131:1099–

112.

112. Sherrard RM, Dixon KJ, Bakouche J, Rodger J, Lemaigre-Dubreuil Y, Mariani J. Differential expression of TrkB isoforms switches climbing fiber-Purkinje cell synaptogenesis to selective synapse elimination. *Dev Neurobiol.* 2009;69:647–62.

113. Letellier M, Bailly Y, Demais V, Sherrard RM, Mariani J, Lohof AM. Reinnervation of late postnatal Purkinje cells by climbing fibers: neosynaptogenesis without transient multi-innervation. *J Neurosci.* 2007;27:5373–83.

114. Ribar TJ, Rodriguiz RM, Khiroug L, Wetsel WC, Augustine GJ, Means AR. Cerebellar defects in Ca^{2+} /calmodulin kinase IV-deficient mice. *J Neurosci.* 2000;20:Rc107.

115. Bosman LW, Hartmann J, Barski JJ, Lepier A, Noll-Hussong M, Reichardt LF, et al. Requirement of TrkB for synapse elimination in developing cerebellar Purkinje cells. *Brain Cell Biol.* 2006;35:87–101.

116. Choo M, Miyazaki T, Yamazaki M, Kawamura M, Nakazawa T, Zhang J, et al. Retrograde BDNF to TrkB signaling promotes synapse elimination in the developing cerebellum. *Nat Commun.* 2017;8:195.

117. Lindvall O. Treatment of Parkinson's disease using cell transplantation. *Philos Trans R Soc Lond Ser B Biol Sci.* 2015;370:20140370.

118. Kordower JH, Freeman TB, Snow BJ, Vingerhoets FJ, Mufson EJ, Sanberg PR, et al. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N Engl J Med.* 1995;332:1118–24.

119. Rylander Ottosson D, Lane E. Striatal plasticity in L-DOPA- and graft-induced dyskinesia; the common link? *Front Cell Neurosci.* 2016;10:16.

120. Piccini P, Lindvall O, Bjorklund A, Brundin P, Hagell P, Ceravolo R, et al. Delayed recovery of movement-related cortical function in Parkinson's disease after striatal dopaminergic grafts. *Ann Neurol.* 2000;48:689–95.

121. Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med.* 2001;344:710–9.

122. Olanow CW, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol.* 2003;54:403–14.

123. Piccini P, Pavese N, Hagell P, Reimer J, Bjorklund A, Oertel WH, et al. Factors affecting the clinical outcome after neural transplantation in Parkinson's disease. *Brain.* 2005;128:2977–86.

124. Roy NS, Cleren C, Singh SK, Yang L, Beal MF, Goldman SA. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. *Nat Med.* 2006;12:1259–68.

125. Bjorklund LM, Sanchez-Pernaute R, Chung S, Andersson T, Chen IY, McNaught KS, et al. Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc Natl Acad Sci U S A.* 2002;99:2344–9.

126. Fukuda H, Takahashi J, Watanabe K, Hayashi H, Morizane A, Koyanagi M, et al. Fluorescence-activated cell sorting-based purification of embryonic stem cell-derived neural precursors averts tumor formation after transplantation. *Stem Cells*. 2006;24:763–71.
127. Bieberich E, Silva J, Wang G, Krishnamurthy K, Condie BG. Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants. *J Cell Biol*. 2004;167:723–34.
128. Parish CL, Parisi S, Persico MG, Arenas E, Minchiotti G. Cripto as a target for improving embryonic stem cell-based therapy in Parkinson's disease. *Stem Cells*. 2005;23:471–6.
129. Xiao B, Ng HH, Takahashi R, Tan EK. Induced pluripotent stem cells in Parkinson's disease: scientific and clinical challenges. *J Neurol Neurosurg Psychiatry*. 2016;87:697–702.
130. Krystkowiak P, Gaura V, Labalette M, Rialland A, Remy P, Peschanski M, et al. Alloimmunisation to donor antigens and immune rejection following foetal neural grafts to the brain in patients with Huntington's disease. *PLoS One*. 2007;2:e166.
131. Morizane A, Li JY, Brundin P. From bench to bed: the potential of stem cells for the treatment of Parkinson's disease. *Cell Tissue Res*. 2008;331:323–36.
132. Ma Y, Feigin A, Dhawan V, Fukuda M, Shi Q, Greene P, et al. Dyskinesia after fetal cell transplantation for parkinsonism: a PET study. *Ann Neurol*. 2002;52:628–34.
133. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med*. 2008a;14:504–6.
134. Kordower JH, Chu Y, Hauser RA, Olanow CW, Freeman TB. Transplanted dopaminergic neurons develop PD pathologic changes: a second case report. *Mov Disord*. 2008b;23:2303–6.
135. Cendelin J, Mitoma H. Neurotransplantation therapy. *Handb Clin Neurol*. 2018;155:379–91.
136. Chen X, Tang TS, Tu H, Nelson O, Pook M, Hammer R, et al. Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 3. *J Neurosci*. 2008;28:12713–24.
137. Boy J, Schmidt T, Wolburg H, Mack A, Nuber S, Bottcher M, et al. Reversibility of symptoms in a conditional mouse model of spinocerebellar ataxia type 3. *Hum Mol Genet*. 2009;18:4282–95.
138. Liu J, Tang TS, Tu H, Nelson O, Herndon E, Huynh DP, et al. Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 2. *J Neurosci*. 2009;29:9148–62.
139. Furrer SA, Waldherr SM, Mohanachandran MS, Baughn TD, Nguyen KT, Sopher BL, et al. Reduction of mutant ataxin-7 expression restores motor function and prevents cerebellar synaptic reorganization in a conditional mouse model of SCA7. *Hum Mol Genet*. 2013;22:890–903.
140. Chort A, Alves S, Marinello M, Dufresnois B, Dornbierer JG, Tesson C, et al. Interferon beta induces clearance of mutant ataxin 7 and improves locomotion in SCA7 knock-in mice. *Brain*. 2013;136:1732–45.
141. Nobrega C, Nascimento-Ferreira I, Onofre I, Albuquerque D, Hirai H, Deglon N, et al. Silencing mutant ataxin-3 rescues motor deficits and neuropathology in Machado-Joseph disease transgenic mice. *PLoS One*. 2013;8:e52396.

142. Rodriguez-Lebron E, Costa Mdo C, Luna-Cancelon K, Peron TM, Fischer S, Boudreau RL, et al. Silencing mutant ATXN3 expression resolves molecular phenotypes in SCA3 transgenic mice. *Mol Ther*. 2013;21:1909–18.
143. Wang HL, Hu SH, Chou AH, Wang SS, Weng YH, Yeh TH. H1152 promotes the degradation of polyglutamine-expanded ataxin-3 or ataxin-7 independently of its ROCK-inhibiting effect and ameliorates mutant ataxin-3-induced neurodegeneration in the SCA3 transgenic mouse. *Neuropharmacology*. 2013;70:1–11.
144. Ramachandran PS, Bhattarai S, Singh P, Boudreau RL, Thompson S, Laspada AR, et al. RNA interference-based therapy for spinocerebellar ataxia type 7 retinal degeneration. *PLoS One*. 2014;9:e95362.
145. Ilg W, Bastian AJ, Boesch S, Burciu RG, Celnik P, Claassen J, et al. Consensus paper: management of degenerative cerebellar disorders. *Cerebellum*. 2014;13:248–68.
146. Ferrucci R, Bocci T, Cortese F, Ruggiero F, Priori A. Noninvasive cerebellar stimulation as a complement tool to pharmacotherapy. *Curr Neuropharmacol* 2017.
147. Benussi A, Koch G, Cotelli M, Padovani A, Borroni B. Cerebellar transcranial direct current stimulation in patients with ataxia: a double-blind, randomized, sham-controlled study. *Mov Disord*. 2015;30:1701–5.
148. Benussi A, Dell’Era V, Cotelli MS, Turla M, Casali C, Padovani A, et al. Long term clinical and neurophysiological effects of cerebellar transcranial direct current stimulation in patients with neurodegenerative ataxia. *Brain Stimul*. 2017;10:242–50.